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Microbial volatile organic compounds in the air of moldy and mold-free indoor environments

Abstract A single-blinded study was performed to analyze whether indoor environments with and without mold infestation differ significantly in microbial volatile organic compounds (MVOC) concentrations. Air sampling for MVOC was performed in 40 dwellings with evident mold damage and in 44 dwellings, where mold damage was excluded after a thorough investigation. The characteristics of the dwellings, climatic parameters, airborne particles and air exchange rates (AER) were recorded. The parameters mold status, characteristics of the interiors and measured climatic parameters were included in the multiple regression model. The results show no significant association between most of the analyzed MVOC and the mold status. Only the compounds 2-methyl-1-butanol and 1-octen-3-ol indicated a statistically significant, but weak association with the mold status. However, the concentrations of the so-called MVOC were mainly influenced by other indoor factors. 2-Methylfuran and 3-methylfuran, often used as main indicators for mold damage, had a highly significant correlation with the smoking status. These compounds were also significantly correlated with the humidity and the AER. The compounds 3-methyl-1-butanol, 2-hexanone, 3-heptanone and dimethyl disulfide were weakly correlated with the recorded parameters, the humidity being the strongest influencing factor. Only 2-methyl-1-butanol and 1-octen-3-ol showed a statistically significant association with the mold status; however, only a small portion (10% in this case) of the total variability could be explained by the predictor mold status; they do not qualify as indicator compounds, because such minor correlations lead to a too excessive part of incorrect classifications, meaning that the diagnostic sensitivity and specificity of these compounds are too low.

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Practical Implications
The assumption that mold infestations might be detected by microbial VOC emissions must be considered with great reservation. The major part of the total variability of the measured MVOC concentrations originates from not known influencing factors and/or from factors not directly associated with the mold status of the dwellings (confounders). More specific and sensitive markers for the assessment of the mold status should be found, if the screening for mold infestations should be performed by volatile organic compounds.

Introduction

The scientific literature on moisture-related problems in buildings (dampness) and health has been reviewed by a Nordic and a European expert group (Bornehag et al., 2001, 2004) and by a North American group (National Academy of Sciences, 2004). The major conclusion is that there is strong evidence that moisture-related problems in buildings increase the risk for health effects among atopics, non-atopics, children and adults both in domestic and public environments. The most important health effects that are associated with

dampness in buildings seem to be airway symptoms, such as wheezing, cough, and asthma. Furthermore, general symptoms and airway infections seem also to be associated with dampness indoors. Relative risks for living or working in damp buildings are in the range of odds ratio (OR) 1.4–2.2. However, the literature is not conclusive in respect of causative agents in such buildings. Suggested causative agents are microbiological agents like mold spores, mites, and organic chemicals from degraded building materials.

Microbial volatile organic compounds (MVOC) are dampness-related agents emitted by microorganisms. These compounds have been discussed to be either noxious agents or at least as useful indicators for mold infestations. The emissions of MVOC by molds and bacteria into the environment have been proven by numerous laboratory trials (Claeson et al., 2002; Gao et al., 2002; Kiviranta et al., 1998; Korpi et al., 1998a; Magan and Evans, 2000; Schleibinger et al., 2005; Sunesson et al., 1995, 1996; Wilkins and Larsen, 1995; Wilkins et al., 2000). It is still unclear which role these compounds play within the microorganism, according to the current state of knowledge, they are not required for microbial metabolism and thus referred to as secondary metabolites. At present, we have no reason to expect toxic effects in humans because of very low-MVOC concentrations (Kreja and Seidel, 2002). However, some compounds have a very unpleasant odor and influence the well-being (Böck, 2001).

It has been discussed for some time whether the MVOC analysis is a suitable method for the detection of concealed mold infestation in indoor environments. Some examiners propose the use of certain MVOC to detect such concealed infestations (Keller, 2001; Lorenz, 2001; Ström et al., 1994; Wessén and Schoeps, 1996). This procedure is thought to be advantageous because MVOC that are constantly emitted by microorganisms can permeate materials and pass leakages, thus enabling the detection of concealed mold infestation e.g. behind wallpaper, paneling and in hollow spaces like wall cavities. However, it must be mentioned here that certain materials like gypsum boards may also be a strong sink of MVOC. Moreover, there is evidence that the emission rates, i.e. the area-specific emission of MVOC per square meter and per hour, are relatively low (Pasanen et al., 1998; Schuchardt et al., 2001). At a given air exchange rate (AER), the indoor air concentrations are very low, usually below the detection limit (Schleibinger et al., 2003). Another disadvantage of the use of MVOC for mold detection is that other chemical and biological sources exist for almost all 'MVOC' (Schleibinger et al., 2004a), particularly cigarette smoke (Newsome et al., 1965; Norman, 1977; Schlotzhauer and Chortyk, 1987), cooking, baking, frying (Anon 2000, Machiels et al., 2003), plant soil, compost bins, and other sources (Wilkins, 1994, 1996). A summary of the use of different MVOC sampling in diagnostic evaluations of buildings is given in the American Industrial Hygiene Association's Field Guide for the Determination of Biological Contaminants in Environmental Samples (AIHA 2005).

Aim of the study

The aim of this field study was to examine whether statistically-confirmed associations exist between the concentration of selected MVOC in indoor air and the mold status of the examined rooms. A further objective was to determine whether other room characteristics not directly associated with mold infestation influence the level of MVOC concentrations. To evaluate the suitability of MVOC for the detection of fungal damage, a field study was performed in 40 apartments with and 44 without mold infestation.

Methods

Study design

The investigation was designed as a single-blinded observational study, i.e. the mycological as well as the MVOC laboratory were blinded in respect to the true mold status of the investigated rooms. The MVOC were analyzed by an accredited laboratory fulfilling the quality criteria according to the German Accreditation Council. Site visits and sampling were performed by staff of the Robert Koch Institute. Sampling was performed between January 2001 and May 2003. The results of the inspections were recorded in standardized protocols recommended for indoor research (Beyer et al., 1997).

The mold status was defined according to the results of the inspection and served as an independent reference standard for the assessment of the analytical and microbiological methods.

Selection of apartments

Information regarding apartments with mold infestation was obtained from the Berlin health authorities. In order to assess the sensitivity of the MVOC method in an unbiased manner, 40 rooms with a broad range of mold-infested areas were included in the study: 11 rooms had a mold-infested area of no more than 0.25 m^2, 10 rooms had a mold-infested area between 0.25 and 1 m^2, and 19 rooms had more than 1 m^2. The mentioned 'small' infestations were included, to show, whether the method is sensitive enough to be a valuable detection method for mold growth. Only apartments were chosen, were a mold infestation was unambiguously confirmed, e.g. by active mold growth on walls, ceilings, windows, tiles, or parts of furniture.

Therefore, measurements were performed mainly in the cooler and/or more humid seasons. The mold infestations were visually assessed during a walk-through inspection by mycologically staff trained in mycology. The results of the walk-through inspection were confirmed in every case by samples from surfaces with Replicate Organism - Detection and Counting (RODAC) plates. The RODAC plate method could prove the fungal infestation; however, in terms of revealing the predominant mold genera, this method was limited, as the plates were frequently completely overgrown. For this limitation, additionally widely-accepted mycological methods were utilized as well to prove the mold status, like active air sampling with two-stage Anderson impactors, probing house-dust samples and settled fungal spores as well. The results of the latter three methods showed a predominance and distinctly higher concentrations of the genera *Aspergillus* and/or *Penicillium* in the samples of the moldy apartments (results published elsewhere (Schleibinger et al., 2004c,d,e), which is a clear indication of active mold growth.

Moldy, musty, or bad odor was not used as a grouping criterion for the mold status. In our study only in 19 mold infested rooms, which were not identical with the 19 apartments with the heaviest mold infestations, such odors were perceived. Chemical-analytical investigation of MVOC seemed to be superior in respect to the (subjective) odor perception, as published odor thresholds are at least two and more orders of magnitude higher compared to the respective detection limits of the analytical method, particularly regarding the here investigated alcohols, ketones, 2-methylfuran and 2-n-pentylfuran. Only dimethyl sulfide and dimethyl disulfide have comparatively low-odor thresholds in the range of 5.9 and 1 $\mu g/m^3$ (Devos et al., 1990; Ruth, 1986; Schiffman et al., 2001).

Forty-four apartments without mold infestation were selected from an indoor study and an environmental field study of the Robert Koch Institute. Only those apartments were included in the category 'apartment without mold infestation', if they were absolutely unsuspicious and in which mold growth was never been observed by the residents. We excluded apartments with previous water and microbial damages, with water stains on walls and/or ceilings, with unpleasant/moldy odor, and where paneling etc. might conceal mold infestation. All apartments were carefully inspected by a walk-through inspection and by mycological sampling as well to check the absence of mold infestation before sampling of MVOC in indoor environments was started.

Sampling and analytic detection of MVOC

The following MVOC in indoor air samples, often considered as predictors for mold damage (Keller, 2001; Lorenz, 2001; Ström et al., 1994; Wessén and Schoeps, 1996), were examined in this study: 2-methylfuran, 3-methylfuran, 2-n-pentylfuran, dimethyl sulfide, dimethyl disulfide, dimethyl sulfoxide, 2-pentanol, 2-methyl-1-butanol, 3-methyl-1-butanol, 1-octen-3-ol, 3-octanol, 1-decanol, 2-hexanone, 2-heptanone and 3-octanone. Sampling was performed in accordance with the VDI (Association of German Engineers) guidelines (VDI 4300 Blatt 6, 2000), 'measuring indoor air contamination – measurement strategies for volatile organic compounds (VOC)' under 'worst case' conditions in closed rooms at least 12 h after last the last airing of the apartment. Over 90% of the air samples were taken on thermal desorption tubes. Two hundred and fifty milligram of Tenax TA served as absorbent. The sampling volume was 2.0 l at an airflow rate of 0.1 l/min. Two parallel samples were taken for each room, and a third tube served as a blind sample. As a result of capacity reasons, activated charcoal tubes (type Anasorb 747, SKC Inc., Eighty Four, PA, USA) had to be used in a small number of rooms. In this case, 120 l of indoor air was pulled through the Anasorb tubes at a flow rate of 2.0 l/min. The Anasorb tubes were desorbed with 2 ml dichloromethane:methanol 95:5. Samples from outdoor air were not taken, as, to our experience, as well as to other authors' results, MVOC concentrations are mostly below the detection limit.

The samples were transferred to the gas chromatography (GC) by an automatic sample injector (TDSA; Gerstel Inc., Mülheim an der Ruhr, Germany). The samples were thermally desorbed at 250°C for 10 min, focused on a refrigerated trap at −60°C and subsequently injected to a GC (HP 5890 series II+, Agilent Technologies, Inc., Santa Clara, CA, USA).

Gas chromatographic separation was achieved on a HP Ultra 2 column (length 50 m, inner diameter 0.21 mm, film thickness 0.33 μm). The following temperature program was selected: 30°C for 0.3 min, followed by 10°C/min to 100°C, then 4°C/min to 130°C, and finally 30°C/min to 300°C. The qualitative and quantitative analysis was performed using a mass spectrometer (HP 5971) in the single ion monitoring mode. The procedure's detection limit is 0.1 $\mu g/m^3$ per compound.

Determining the air exchange rate

The AER was estimated from the decrease rate of the measured CO_2 concentrations in the investigated rooms according to the VDI guidelines (VDI 4300 Blatt 7, 2001) by nonlinear regression (Laußmann et al., 2002).

Climatic parameters of the indoor environment

To determine the impact of the room air temperature and humidity on the MVOC concentrations a

temperature and humidity measurement device (Testostor 171-3; Testo Co., Lenzkirch, Germany) was set up in the test room 24 h before MVOC sampling.

Particle concentration

For the determination of airborne particles a laser counter (forward light scattering, type Met One 237B; Met One Inc., Grants Pass, OR, USA) was used. Six size ranges were recorded: >0.3, 0.5, 0.7, 1, 2 and >5 μm.

Inspection protocol

The inspection protocol was written in accordance with the guideline 'quality assurance in environmental medicine' (Beyer et al., 1997). The following descriptors and measured parameters were used for statistical analysis:

- Residential area: housing area with grassy plains (yes/no).
- Building: type of heating, smoking status of the apartment, smoking in other rooms of the apartment during sampling.
- Sampling room: room volume, room's purpose, air exchange rate, room temperature, and humidity during the last 24 h before sampling, type of floor covering, data on room furnishings, the presence of chip board furniture or structural enclosures made of chip board, number of plants, visible mold infestation.

Statistical methods

Univariate statistics. All recorded room characteristics, which were included in statistical analyzes, were dichotomized and coded 0/1 (Table 1). ANOVA was performed with the log-concentrations as the dependent and the mold status as the independent variable. For compounds where the hypothesis of log-normality was rejected (see above), the non-parametric Mann–Whitney test was used (Table 2). For MVOC, which were below the detection limit in more than 90% of all cases, no further statistical analysis was performed. The latter applies for the following compounds: 2-pentanol, 3-octanol, 1-decanol, 3-octanone, and dimethyl sulfoxide. For testing possible associations between other room characteristics as the independent variables and the transformed concentrations of 10 MVOC ANOVA – in the case of log-normally distributed MVOC – or the Mann–Whitney test was used (Table 3a). If the independent variables were measured on a continuous scale, like the AER (h^{-1}), the water content of the room air ($g\ m^{-3}$), or the concentration of fine particles ($d_{ae} < 1$ μm/l), these variables were also log-transformed before the analysis, because the distributions of the measured values were right-skewed, and log-normally distributed. For these three parameters the hypothesis for log-normality was not rejected by the Kolmogorov–Smirnov test.

It was also tested, whether the presence of plants is an influencing factor on MVOC concentrations. In general, in our study plants appeared in relatively small numbers in the examined rooms. The range in moldy rooms was 0–11 plants and in mold-free rooms 0–13 plants. To avoid the logarithmic calculus of a zero frequency, an absolute term of three of the eight was added to the counted number of green plants, as proposed by Sachs (1999). The so-corrected plant count was then divided through the room volume to get the plant density; this ratio was subsequently log-transformed. Hypothesis of log-normality was then accepted by the Kolmogorov–Smirnov test.

Table 1 Overview of the investigated potential categorical and metric $\log_{10}$-transformed, influencing factors on indoor air concentrations of selected MVOC with short-symbol and denotiation (code)

Influencing factor	Symbol	Denotiation (encoding item)		n_0/n_1	n
Categorical					
Visible mold infestation	MI	no (0)	yes (1)	44/40	84
Housing area with grassy plains	HA	no (0)	yes (1)	37/47	84
Main function of the room	MF	mainly working (0)	living only (1)	14/70	84
Furnishing of the room	FR	no (0)	yes (1)	4/80	84
Textile floor coverings	TC	non/partially cov. (0)	wall to wall (1)	41/43	84
Particle board furniture	PB	absent (0)	present (1)	43/41	84
Household with smoker(s)	HS	no (0)	yes (1)	66/18	84
Smoking in neighboring room	SS	no (0)	yes (1)	73/11	84
Gas heating/cooking devices	GH	no (0)	yes (1)	73/11	84
CO_2 level (24 h average)	CO_2	$\leq$ 1000 ppm (0)	>1000 ppm (1)	58/26	84
Sorbent	AM	Anasorb 747 (0)	Tenax TA (1)	3/81	84
Metric ($\log_{10}$-transformed)					
Absolute humidity (24 h) (g/m^3)	AH				84
Air exchange rate (h^{-1})	AC				84
Indoor particle concentration l^{-1} air (d_{ae} <1 μm)	PC				83
Density of foliage plants in the room $[(n + 3/8)/V_R]$	PD				84

n_0, number of rooms with encoding item '0'; n_1, number of rooms with encoding item '1'; d_{ae}, aerodynamic diameter; n, number of foliage plants in the room; V_R, room volume.

Table 2 Percentiles and geometric mean values of the indoor air concentrations of selected MVOC in moldy and non-moldy homes. For statistically significant differences in the median concentrations between moldy and non-moldy homes, estimated with ANOVA or the Mann–Whitney test, respectively, the achieved significance levels (*P*-values) are given

Compound	Visible mold	Percentile					GM	*P*-value
		5th	10th	50th	90th	95th		
2-methylfuran	Yes	0.30	0.40	1.16	3.76	4.19	1.21	<0.001
	No	0.12	0.20	0.65	2.05	3.50	0.65	
3-methylfuran	Yes	<	0.10	0.32	0.70	1.20	0.32	<0.001
	No	<	<	0.20	0.48	1.12	0.17	
2-n-pentylfuran	Yes	0.40	0.51	1.19	2.21	3.25	1.09	<0.05
	No	0.10	0.14	0.85	2.25	2.63	0.73	
Dimethyl sulfide	Yes	<	<	<	0.84	1.25	0.15	<0.010
	No	<	<	<	0.30	1.05	0.08	
Dimethyl disulfide	Yes	<	<	0.10	0.49	2.90	0.15	<0.001
	No	<	<	<	0.20	0.20	0.07	
Dimethyl sulfoxide	Yes	<	<	<	0.10	0.50	0.06	n.s.
	No	<	<	<	<	<	0.06	
2-pentanol	Yes	<	<	<	<	1.16	0.06	n.s.
	No	<	<	<	0.28	0.60	0.07	
3-methyl-1-butanol	Yes	<	<	0.93	1.99	2.68	0.41	<0.05
	No	<	<	0.23	1.48	1.88	0.22	
2-methyl-1-butanol	Yes	<	<	0.08	1.18	1.70	0.16	<0.01
	No	<	<	<	0.50	0.53	0.08	
1-octen-3-ol	Yes	<	<	<	0.49	1.37	0.13	<0.001
	No	<	<	<	0.28	0.38	0.06	
3-octanol	Yes	<	<	<	<	<	<	n.s.
	No	<	<	<	<	<	<	
1-decanol	Yes	<	<	<	<	0.53	0.06	n.s.
	No	<	<	<	0.13	0.59	0.06	
2-hexanone	Yes	0.21	0.31	0.60	1.29	1.30	0.57	n.s.
	No	<	<	0.58	1.55	2.10	0.47	
2-heptanone	Yes	0.31	0.61	1.40	2.75	3.19	1.21	n.s.
	No	0.12	0.24	1.05	3.30	4.18	0.96	
3-octanone	Yes	<	<	<	<	<	0.05	n.s.
	No	<	<	<	<	<	0.05	

n.s., statistically non significant differences between estimated median values; <, values below the detection limit of 0.1 $\mu g/m^3$.

Pearson's correlation coefficients (P's R) were determined between MVOC with log-normally distributed concentrations and these four variables (Table 3b). For the non-lognormally distributed MVOC concentrations Spearman's rank correlation coefficients (Sp's R) were calculated instead (Table 3b).

Multiple regression analysis. Following the univariate analysis multiple linear regression was performed with the log-transformed concentrations of each (M)VOC (see e.g. Hoffmann et al., (2000). The regression model included as independent variables the mold status of the examined room together with the dichotomized categorical room characteristics and the log-transformed metric parameters [climatic parameters of the indoor environment, AER, concentration of particles in indoor air ($d_{ae} < 1$ μm/l) and plant density as possible predictors for the (M)VOC concentrations (Table 1)]. The type of sorbent (Tenax TA vs. Anasorb 747) was also included in the analysis as possible predictor.

In the multiple regression analysis, the procedure 'stepwise' with a pair-wise case elimination was applied. The estimated regression parameters of the final models were then back-transformed to the normal scale. The independent contributions of all these factors to the total explained variance (R^2) were estimated in this regression model and are tabulated with the regression coefficients in Table 4.

To verify the adequateness of the multiple regression model, for each compound differences between the measured and calculated concentrations (i.e. the residuals) were generated. The empirical distributions of the residuals were examined and tested for normality with the Kolmogorov–Smirnov test again. Hypothesis of normality was only rejected for the compounds dimethyl sulfide, 2-methyl-1-butanol and 2-hexanone.

The goodness of fit of the multiple regression model for the individual MVOC is given by the multiple coefficient of determination R^2_{mult}. This coefficient describes the percentage of the overall variance of a compound, which can be explained by the mutual influence of all significant predictors. This explained overall variance can be split into single variance proportions (R^2), which explain the independent contribution of each of the significant predictors and give as their sum R^2_{mult}. In this way, it can be shown, which factors (predictors) have a higher and which have a lower influence on indoor air concentrations of individual MVOC. A probability value of $P < 0.05$ (two-sided) for all statistical analyzes was *a priori* considered as statistically significant. All calculations were performed with the statistical package SPSS 11.5.

Results

Univariate data analysis

Frequency of MVOC occurrence in moldy and mold-free homes. The results of the frequency analysis (χ^2-test) show that several MVOC, considered as typical for mold growth, were found in relative high frequencies in both homes with and without mold growth (detection limit 0.1 $\mu g/m^3$). For these compounds no statistical significant differences in the distribution frequencies could be found. On the other hand some MVOC were only rarely detected in mold-free homes and, surprisingly, also rarely in moldy homes. Only the compounds dimethyl disulfide, 2-methyl-1-butanol and 1-octen-3-ol were found significantly more frequently in moldy homes. It should be noted, too, that these compounds were not determined in all moldy apartments. The frequencies of these MVOC range from 73% for dimethyl disulfide to 50% and 48% for 2-methyl-1-butanol and 1-octen-3-ol, respectively.

Table 3a Categorical influencing factors, frequency of occurrence (*n*), and geometric means (GM) of the measured room air concentrations of selected MVOC

		2-methyl-furan		3-methyl-furan		2-n-pentyl-furan		Dimethyl sulfide		Dimethyl disulfide		3-methyl-1-butanol		2-methyl-1-butanol		1-octen-3-ol		2-hexanone		2-heptanone	
MVOC influencing factor	*n*	GM	*P*-value	GM	*P*-value	GM	*P*-value	GM	*P*-value	GM	*P*-value	GM	*P*-value	GM	*P*-value	GM	*P*-value	GM	*P*-value	GM	*P*-value
Categorical																					
Housing area																					
Without grassy plains	37	0.99	n.s.	0.26	n.s.	0.88	n.s.	0.11	n.s.	0.10	n.s.	0.34	n.s.	0.11	n.s.	0.09	n.s.	0.50	n.s.	1.24	n.s.
With grassy plains	47	0.79		0.22		0.89		0.10		0.10		0.27		0.11		0.09		0.52		0.96	
Main function of the room																					
Mainly working	14	0.45	<0.01	0.12	<0.01	0.39	<0.001	0.05	<0.05	0.05	<0.01	0.06	<0.001	0.06	<0.05	0.06	<0.05	0.44	n.s.	0.57	<0.01
Living only	70	0.99		0.26		1.04		0.12		0.11		0.41		0.12		0.10		0.53		1.22	
Furnishing																					
Unfurnished (empty)	4	0.22	0.001	0.07	<0.01	0.26	<0.01	0.05	n.s.	0.06	n.s.	0.08	n.s.	0.05	n.s.	0.08	n.s.	0.42	n.s.	0.76	n.s.
Furnished room	80	0.93		0.25		0.94		0.11		0.10		0.32		0.11		0.09		0.52		1.09	
Floor coverings																					
Uncovered/partially covered	41	0.81	n.s.	0.21	n.s.	0.75	n.s.	0.09	n.s.	0.09	n.s.	0.20	<0.05	0.08	n.s.	0.09	n.s.	0.46	n.s.	1.07	n.s.
Wall-to-wall	43	0.93		0.26		1.03		0.12		0.10		0.43		0.15		0.10		0.58		1.08	
Particle board furniture																					
Absent	43	0.70	<0.05	0.19	<0.05	0.64	<0.001	0.09	n.s.	0.08	n.s.	0.21	<0.05	0.09	n.s.	0.08	n.s.	0.44	n.s.	1.01	n.s.
Present	41	1.09		0.30		1.24		0.13		0.13		0.44		0.13		0.10		0.61		1.15	
Smoker status (dwelling)																					
Never smoker (ref. group)	55	0.58	–	0.16	–	0.84	–	0.10	–	0.08	–	0.36	–	0.12	–	0.09	–	0.49	–	1.04	–
Smoker	18	1.39	<0.001	0.37	<0.001	0.89	n.s.	0.09	n.s.	0.10	n.s.	0.15	n.s.	0.10	n.s.	0.09	n.s.	0.54	n.s.	1.02	n.s.
Smoking in neighboring room	11	3.21	<0.001	0.81	<0.001	1.12	n.s.	0.12	n.s.	0.29	<0.001	0.33	n.s.	0.08	n.s.	0.09	n.s.	0.61	n.s.	1.39	n.s.
CO_2 level (24-h average)																					
≤ 1000 ppm	58	0.73	<0.01	0.19	0.001	0.80	n.s.	0.08	<0.01	0.09	<0.05	0.27	n.s.	0.10	n.s.	0.08	n.s.	0.50	n.s.	0.95	0.050
>1000 ppm	26	1.28		0.37		1.10		0.19		0.14		0.36		0.13		0.11		0.55		1.42	
Sorbent																					
Anasorb 747	3	0.34	n.s.	0.08	<0.05	0.34	n.s.	–	–	0.07	n.s.	0.05	n.s.	0.08	n.s.	0.07	n.s.	0.22	n.s.	0.70	n.s.
Tenax TA	81	0.90		0.24		0.92		0.10		0.10		0.32		0.11		0.09		0.53		1.09	
Gas heating/cooking devices																					
Absent	73	0.84	n.s.	0.23	n.s.	0.83	n.s.	0.10	n.s.	0.10	n.s.	0.27	n.s.	0.11	n.s.	0.09	n.s.	0.49	n.s.	1.04	n.s.
Present	11	1.07		0.29		1.30		0.14		0.09		0.53		0.12		0.10		0.74		1.37	

Results of the univariate statistical analysis (ANOVA) or Mann–Whitney test are given as *P*-value (significance level).
n.s., statistically non significant differences between geometric means.

Concentration differences of MVOC in respect to moldy and mold-free homes. Subsequently to the frequency analysis with the χ^2-test an ANOVA or Mann–Whitney test was performed concerning the concentration differences of MVOC between moldy and mold-free homes. Of the 15 compounds tested eight MVOC showed significantly higher geometric mean concentrations in moldy homes (Table 3a,b).

Besides the mold status other factors (see Table 1) were tested with ANOVA or correlation analysis for their potential influence or association on the MVOC concentrations. The evaluation showed that also some of the recorded room characteristics had an impact on the MVOC concentrations (Table 4 for the categorical and metric room descriptors). This applies e.g. for the categorical factors 'main function of the room', 'average CO_2 level', 'particle board furniture' and 'furnishing'. The factor 'floor covering' had an impact only on the concentration of 3-methyl-1-butanol. Especially the smoking status of the homes strongly influenced the concentrations of 2- and 3-methylfuran (Table 4). Many MVOC had a positive correlation coefficient (P's *R*) or (Sp's *R*) with the metric factors 'absolute humidity', and some MVOC with the factor 'density of foliage plants in the room'. 2- and 3-methylfuran and dimethyl disulfide were positively correlated with the concentration of airborne sub-micron particles. A number of MVOC were negatively correlated with the AER (Table 4).

Multiple regression analysis

Included and persisting influencing factors. For every MVOC already tested in the univariate analysis the persisting factors were determined with the multiple linear regression model. Only those 10 compounds for which significant correlations have been found are presented (Table 4). The analysis showed a significant association with the mold status of the apartment only for the compounds 2-methyl-1-butanol ($P < 0.01$) and 1-octen-3-ol ($P < 0.01$). For the compound dimethyl disulfide the correlation was only significant, if the analysis was limited to the group of

Table 3b Results of the univariate correlation analysis (Pearson's correlation coefficient, P's *R*, or Spearman's rank correlation coefficient, Sp's *R*) between room air concentrations and metric, $\log_{10}$-transformed, influencing factors

MVOC influencing factor	*n*	2-methylfuran		3-methylfuran		2-n-pentyl-furan		Dimethyl sulfide		Dimethyl disulfide		3-m-1-butanol		2-methyl-1-butanol		1-octen-3-ol		2-hexanone		2-heptanone	
		P's *R*	*P*-value	P's *R*	*P*-value	P's *R*	*P*-value	Sp's *R*	*P*-value	Sp's *R*	*P*-value	Sp's *R*	*P*-value	Sp's *R*	*P*-value	Sp's *R*	*P*-value	P's *R*	*P*-value	P's *R*	*P*-value
Metric ($\log_{10}$-transformed)																					
Absolute humidity (g/m^3)	84	0.392	<0.001	0.376	<0.001	0.628	<0.001	0.253	n.s.	0.322	<0.01	0.345	0.001	0.132	n.s.	0.408	<0.001	0.531	<0.001	0.631	<0.001
Air exchange rate (h^{-1})	84	−0.440	<0.001	−0.368	0.001	−0.494	<0.001	−0.230	<0.05	−0.196	<0.05	−0.020	n.s.	−0.113	n.s.	−0.213	n.s.	−0.366	0.001	−0.220	<0.05
Indoor particle conc. (l^{-1})	83	0.550	<0.001	0.571	<0.001	−0.043	n.s.	0.063	n.s.	0.474	<0.001	−0.056	n.s.	0.055	n.s.	0.129	n.s.	−0.047	n.s.	0.106	n.s.
Density of foliage plants in the room	84	0.243	<0.05	0.202	n.s.	0.274	<0.05	0.134	n.s.	0.265	<0.01	0.242	<0.05	0.177	<0.05	0.052	n.s.	0.159	n.s.	0.258	<0.05

Number of measurements (*n*) and statistical significance level (*P*-values).
n.s., no statistically significant correlation was found.

non-smoker apartments (Schleibinger et al., 2004b). Room characteristics like smoking status (recorded as smoker apartment or as smoking in adjacent rooms of the apartment during sampling), room furnishings, room's purpose, room climate conditions, and room humidity had highly significant associations with the indoor air concentrations of individual MVOC. Some compounds also showed a minor association with the number of plants in the tested room or with the amount of greenery near the apartment. High multiple coefficients of determination (R^2_{mult}) were observed with alkylfurans. For the methylfurans the predictors smoker apartment, smoking in adjacent rooms and the number of airborne particles $< 1\ \mu$m explained up to 50% of the total variance. With 2-n-pentylfurane, the predictor absolute humidity explained 35% and the AER 16% of the total variance. The main predictor for dimethyl disulfide was the concentration of airborne particles $< 1\ \mu$m (22% explained variance) with the predictor 'average CO_2 concentration > 1000 ppm' explaining almost 16% of the variance of this compound.

For the compounds 2-methyl-1-butanol and 1-octen-3-ol the predictor mold infestation explained only about 10% of the overall variance. These two MVOC were the only compounds for which a statistically significant effect of the predictor 'mold infestation' persisted in the multiple regression model, whereas for the other so-called MVOC no statistically significant influence of the mold infestation remained.

Discussion

In practice MVOC detection methods are already used for the aim of discovering concealed microbial damage without the attempt having been made to sufficiently validate this procedure with regards to its informational value. It was presumed that the presence of these so-called MVOC is highly specific for microbial contamination (Wessén and Schoeps, 1996). But a shortcoming of the MVOC method was the lack of information about concentrations and the spectra of MVOC in mold-free control homes (Pasanen, 2001, Wilkins, 2002). As the sensitivity and the specificity of the MVOC method were not evaluated so far, a nearly equal number of clearly non-infested homes were included in the study presented here.

The main result of the assessment of moldy and mold-free homes was, that several indoor parameters influenced the concentrations of these MVOC, and that the mold status either did not play a decisive role or did not contribute to the MVOC concentration at all. Only for the compounds 2-methyl-1-butanol and 1-octen-3-ol did mold status remain a statistically significant impact factor in the multiple regression model. Even

Table 4 Results of the multiple regression analysis for selected MVOC: persistent influencing factors, adjusted back-transformed regression coefficients with 95% confidence intervals; explained proportion of the total variance (R^2) of the single significant descriptor and the achieved statistical significance level (P-value) are presented

Influencing factor	Symbol	Backtransformed adjusted regression coefficients	95% CI		R^2	P-value
2-methylfuran						
Intercept		0.0025	0.00052	0.012		<0.001
Furnishing	FR	2.26	1.52	3.36	0.068	<0.001
Household with smoker(s)	HS	1.93	1.53	2.45	0.084	<0.001
Smoking in neighboring room	SS	3.64	2.61	5.08	0.283	<0.001
Gas heating/cooking devices	GH	1.42	1.10	1.83	0.012	<0.01
Sorbent	AM	2.12	1.35	3.34	0.033	<0.01
Absolute humidity (g/m^3)	AH	0.83	0.53	1.14	0.103	<0.001
Air exchange rate (h^{-1})	AC	−0.38	−0.50	−0.26	0.134	<0.001
Indoor particle conc. (l^{-1})	PC	0.16	0.05	0.27	0.100	<0.01
Density of foliage plants in the room	PD	0.08	0.01	0.14	0.028	<0.05
R^2_{mult}					0.845	<0.001
3-methylfuran						
Intercept		0.00055	0.000086	0.0036		<0.001
Furnishing	FR	1.92	1.22	3.02	0.049	<0.001
Household with smoker(s)	HS	1.86	1.42	2.43	0.080	<0.001
Smoking in neighboring room	SS	3.24	2.22	4.73	0.255	<0.001
Gas heating/cooking devices	GH	1.47	1.1	1.96	0.015	<0.01
CO_2 level (24-h average)	CO_2	1.10	1.00	1.21	0.041	<0.05
Sorbent	AM	2.40	1.43	4.01	0.045	<0.01
Absolute humidity (g/m^3)	AH	0.86	0.52	1.21	0.105	<0.001
Air exchange rate (h^{-1})	AC	−0.26	−0.40	−0.12	0.078	<0.001
Indoor particle conc. (l^{-1})	PC	0.18	0.05	0.31	0.121	<0.01
R^2_{mult}					0.789	<0.001
2-n-pentylfuran						
Intercept		0.0013	0.00037	0.0046		<0.001
Furnishing	FR	2.89	1.65	5.04	0.082	<0.001
Sorbent	AM	2.90	1.52	5.54	0.048	<0.01
Absolute humidity (g/m^3)	AH	1.71	1.27	2.14	0.345	<0.001
Air exchange rate (h^{-1})	AC	−0.41	−0.58	−0.24	0.164	<0.001
R^2_{mult}					0.639	<0.001
Dimethyl sulfide						
Intercept		0.012	0.002	0.071		<0.001
CO_2 level (24-h average)	CO2	1.484	1.205	1.827	0.157	<0.001
Absolute humidity (g/m^3)	AH	0.854	0.053	1.655	0.055	<0.05
R^2_{mult}					0.212	<0.001
Dimethyl disulfide						
Intercept		1.48E-04	0.000	0.002		<0.001
Furniture with particle board	PB	1.483	1.040	2.111	0.049	<0.05
Absolute humidity (g/m^3)	AH	0.839	0.204	1.475	0.078	0.010
Indoor particle conc. (l^{-1})	PC	0.451	0.286	0.617	0.225	<0.001
Density of foliage plants in the room	PD	0.164	0.026	0.302	0.055	<0.05
R^2_{mult}					0.407	<0.001
2-methyl-1-butanol						
Intercept		0.057	0.038	0.087		<0.001
Visible mold infestation	MI	1.435	1.119	1.841	0.096	<0.01
Textile coverings	TC	1.754	1.067	2.877	0.060	<0.05
R^2_{mult}					0.156	<0.001
3-methyl-1-butanol						
Intercept		0.003	0.000	0.029		<0.001
Main function of the room	MF	6.383	2.818	14.490	0.204	<0.001
Textile coverings	TC	2.511	1.399	4.498	0.078	<0.01
Absolute humidity (g/m^3)	AH	1.184	0.112	2.257	0.074	<0.05
R^2_{mult}					0.356	<0.001
1-octen-3-ol						
Intercept		0.006	0.001	0.024		<0.001
Visible mold infestation	MI	1.303	1.081	1.570	0.103	0.001
Absolute humidity (g/m^3)	AH	1.139	0.471	1.806	0.139	<0.01
R^2_{mult}					0.242	<0.001
2-hexanone						
Intercept		3.9E-03	0.001	0.019		<0.001
Adsorbent medium	AM	2.624	1.083	6.356	0.039	<0.05

Table 4 (Continued)

Influencing factor	Symbol	Backtransformed adjusted regression coefficients	95% CI		R^2	*P*-value
Absolute humidity (g/m^3)	AH	1.562	0.965	2.159	0.264	<0.001
Air exchange rate (h^{-1})	AC	−0.285	−0.524	−0.046	0.083	<0.05
R^2_{mult}					0.386	<0.001
2-heptanone						
Intercept		0.035	0.009	0.132		<0.001
Housing area	HA	0.665	0.491	0.902	0.034	<0.01
Absolute humidity (g/m^3)	AH	1.920	1.390	2.450	0.389	<0.001
Density of foliage plants in the room	PD	0.167	0.044	0.290	0.061	<0.01
R^2_{mult}					0.484	<0.001

Additionally for each compound the multiple coefficient of determination, R^2_{mult} – the proportion of the explained overall variance of the appropriate MVOC-concentration, is given.

then the percentage of the explained total variance by this predictor was relatively low (about 10%, see Table 4).

This result means that 90% of the variance is caused by other parameters and that the range of the respective results obtained in moldy and in mold-free homes overlap to such a degree, that the mold status cannot be concluded by an analytical MVOC result.

It has to be highlighted, that MVOCs mainly originate from fresh and metabolically active microbial contaminations, and to a much lesser extend from microbial activity in the past. Some MVOCs like furans offgas very quickly, while other MVOCs like 1-octen-3-ol with a higher boiling point offgass slower. Thus a correlation of 1-octen-3-ol with the mold status, but no correlations with e.g. furans might point to older mold damages. Regarding the present study it should not be overlooked, that the absence of MVOCs at least in some moldy apartments might have been caused by dry conditions of moldy materials, which means, that offgassing has already taken place by the time sampling was performed. In this present study moisture meters to determine the dampness of building material were not used, to assess the actual growth conditions, but three independent microbial sampling methods used in parallel indicated presence of fungal activity.

Other examiners also found relatively high MVOC concentrations of up to 1 μg/m^3 in apartments not infested with mold (Elke et al., 1999). According to Pasanen et al. (1998) the contribution of the MVOC from mold-infested sites to indoor air concentrations of MVOC is very low and will not lead to higher concentrations in moldy homes. Therefore, the minor concentration differences between apartments with and without mold infestation and the masking effect of other factors impair the indicator function of MVOC. Miller et al. (1988) have already proven, that the MVOC 3-methyl-1-butanol, 2-hexanone and 2-heptanone hardly correlated with parameters considered as indicators for mold damage. The authors concluded that there must be other sources for these compounds, and the MVOC method is possibly only able to detect extremely severe mold infestations.

Statistical evaluation of the results of the present study showed a highly significant association between the smoking status (non-smoker flat, smoker flat, smoking during sampling in neighboring rooms) and the concentrations of some MVOC. This was especially the case for methylfuranes, which are known to originate in large amounts during the pyrolysis of organic materials (Bierbach et al., 1995) like wood (Barrefors and Petersson, 1995; Barrefors et al., 1996; Ingemarsson et al., 1998; Lemieux et al., 2004), but also of tobacco products (Hoffmann and Hoffmann, 1998; Newsome et al., 1965; Norman, 1977; Schlotzhauer and Chortyk, 1987; Xie et al., 2003). According to Anon (2000) cigarette smoke, wood smoke, and meat cooking are considered as main sources for furans in indoor environments. Especially dimethyl disulfide previously considered as highly specific for molds was also found in tobacco smoke (Blomberg and Widmark, 1975). The statistically significant correlation between the concentration of this compound and the particle concentration in the sub-micron range supports the hypothesis that the presence of dimethyl disulfide is at least partly caused by smoking and other pyrolytic processes like cooking or heating dishes, and not exclusively linked with the mold status. Even if smoking is not permitted in the rooms to be tested (which was not performed), one can never exclude, that persons smoke in neighboring rooms or apartments.

Many other experimental studies and field examinations also concluded that MVOC are not specific for microbial growth, but have a number of other sources. It was shown in laboratory experiments that almost all MVOC examined in this study are produced during the heating of animal tissue (Weber and Spleiß, 1997). Furthermore 'MVOC' develop during other processes like cooking, baking, and frying (Elmore et al., 2000; Machiels et al., 2003; Oruna-Concha et al., 2002). Man himself is a source of

MVOC, too. This was proven e.g. for dimethyl sulfide, which was found in the exhalation air of healthy persons in a concentration range between 15 and 20 ng/l (Kaji et al., 1978; Suarez et al., 2000; Tangerman et al., 1983). Many plants also emit a wide spectrum of compounds considered as MVOC like alcohols (e.g. 2-methyl-1-butanol, 1-octen-3-ol, 3-octanol, 3-methyl-1-butanol, 1-octanol) and ketones (e.g. 3-octanone, König et al., 1995; Overton and Manura, 1999). Other biological and chemical sources of MVOC are given by Schleibinger et al., 2004a).

Not only chemical sources, but also other factors including absolute humidity, AER and sometimes even plant density, i.e. factors that are not directly associated with microbiological infestation, were proven to have an impact on MVOC concentrations in this study. For example, the effect of humidity on the MVOC concentration was also confirmed by Korpi et al. (1998b).

The MVOC method is also questioned by reports about the poor congruence between the results of laboratory and field studies. Theoretical considerations by Pasanen et al. (1998), Schuchardt et al. (2001, 2005) have demonstrated, that the low specific emission rates of molds found in laboratory trials would lead to barely detectable indoor air concentrations.

Conclusion

Determining MVOC in indoor air does not yet seem to be suitable for an accurate detection of microbial damages in indoor environments, as the study presented here found only small (if any) associations between visible mold-infested areas (and confirmed active mold growth) and the MVOC investigated here. The associations between hidden mold growth and MVOC in indoor environments are expected to be even weaker. This can be explained not only by the low-emission rates of molds, but also by certain influencing and confounding factors. If microbially produced VOC should be used for disclosing mold infestations in future, more specific MVOC have to be found, with which other influencing or confounding factors regarding the mold status do not play a decisive role. As emission rates regarding more specific MVOC are expected to be very low, those detection methods would have to be very sensitive.

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